Effects of Natural Products on the Recurrence-Free Survival of Spontaneous Mammary Tumours and Their Participation in Inhibition of Secondary Tumours by Whole-body Hyperthermia in SHN Mice.

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Abstract: Based on the anti-mammary tumour effects of natural products, Gold Banded Lily (Lilium auratum Lindl), Chinese Milk Vetch (Astragalus sinicus L), Bitter Melon (Momordica charantia L) and Ginger Rhizome (Zingiber officinale Rose), we examined the effects of these natural products on the recurrent period, the period between the surgical removal of the first tumour and the appearance of new tumour, and their participation in the inhibition of the growth of the new tumour by whole-body hyperthermia with glucose (GW). Free access to Bitter Melon in drinking water (0.5%) elongated significantly the recurrent period and stimulated somewhat the GW inhibition of tumour growth. But the other 3 agents had little effect on the recurrent period and nullified the effects of GW. These findings stress the importance of the therapeutic history of the patient in the efficient application of WBH.

Key Words: tumour, mice, natural products, whole-body hyperthermia (WBH).

Introduction

Gold Banded Lily is known as an antitussive, sedative and anti-pyretic agent. Chinese Milk Vetch is used as an antipiretic and diuretic agent. These plants contain guadino compounds, of which antitumour role was examined in this laboratory1). Bitter Melon, rich in L-ascorbic acid, has an anti-poison, antipiretic and anti-tumour properties. Ginger Rhizome is used as an antipiretic and anti-inflammatory agent.

We have recently found that these 4 natural products significantly suppressed the development of spontaneous mammary tumours in SHN mice2-3). Then, we examined their adjuvant effects on recurrence-free survival.

The whole-body hyperthermia (WBH) began with the fever therapy induced by administering erysipelas bacteria4). Currently, 3 types of technology are applied; direct skin contact, externally
applied power absorption and extracorporeal perfusion, and several apparatuses have been devised, of which progress is briefly reviewed\(^5\)\(^6\). Recently, far-infrared ray (FIR) has been applied as an effective heat source of WBH\(^7\)\(^8\) and combined treatment with biological therapy appears to be measurable benefit of WBH\(^7\)\(^9\)\(^10\).

Recent study in this laboratory has revealed that the inhibition by whole-body hyperthermia (WBH) with FIR of the growth of mammary tumour was much stimulated by the simultaneous administration of glucose\(^11\). Therefore, the effects of this therapeutic system in combination with the above 4 natural products on the growth of secondary tumour were further studied.

**Materials and Methods**

**Animals**

A high mammary tumour strain of SHN/Mei female mice maintained in our laboratory\(^12\)\(^–\)\(^14\) was used. Throughout the experiments, the animals were kept in plastic cages (18 X 35 X 15cm) with wood shavings (M size: CLEA JAPAN, Tokyo, Japan), 4-5 mice per cage, in a windowless animal room, which was air-conditioned (22-23°C and 55-75% relative humidity) and artificially illuminated (14 hours of light from 0500 to 1900 h), and provided with a commercial diet (Lab MR Breeder: Nihon Nosan Kogyo KK, Yokohama, Japan).

**Test samples**

Dry matter of the roots of Gold Banded Lily (*Lilium auratum Lindl*), the seeds of Chinese Milk Vetch (*Astragalus sinicus L*), the fruits of Bitter Melon (*Momordica charantia L*) and the roots of Ginger (*Zingiber officinale Rosc*) were extracted repeatedly with hot water and dried in vaccuo. The dried extracts were dissolved with tap water at a concentration of 0.5% for the first 3 samples. However, the concentration of Ginger was 0.125%, since the mice hardly touched it at higher concentrations and so lost body weight.

**Whole-body hyperthermia (WBH)**

WBH was performed in an insulated room (100 X 190 X 225cm) lined with steel panels and polyurethane. Room temperature was controlled at 37-42°C by a far-infrared ray (FIR) panel in the ceiling. The distance from the panel to the cages was approximately 1 m. The duration of WBH was 3 hours, and the body temperature of mice, which was measured by the probe inserted in the rectum and connected to the recorder\(^15\), was maintained at 39-41°C during WBH. All procedures were the same as detailed previously\(^11\).

**Hyperglycemia**

Glucose (Wako Pure Chemical Industries Ltd., Osaka, Japan) diluted with physiological saline (Otsuka Seiyaku, Tokyo, Japan) was injected intraperitoneally (6 mg/g body weight) 30 min before WBH.
Treatments

Mice at 2 months of age were divided into five groups; one control and four experimentals, Lily, Milk Vetch, Bitter Melon and Ginger groups. The control received tap water and the experimentals each test sample *ad libitum* in drinking water. All mice were checked for palpable mammary tumours twice a week. The first tumours were removed surgically when they reached approximately 6 mm in size, the geometric mean of the two major diameters, and mice continued to be checked for palpable new tumours, secondary tumours. Mice which developed new tumours at the surgical bed of the first tumours were not used. When the secondary tumour reached approximately 10 mm, the test sample was changed to tap water, the mice received a single trial of WBH plus glucose (GW) and tumour size was checked every day until 9 days after GW. Some control mice were untreated, while the remainder were given GW (the control and control + GW groups, respectively). All mice were killed by decapitation under light ether anaethesia after the last measurement of tumour size on day 9.

Growth rate of mammary tumours

Mammary tumour size was measured daily and the % change of tumour size calculated by the following formula was used as an index of the tumour growth rate:

\[
\frac{\text{Tumour size on the respective day} - \text{Tumour size on day 0}}{\text{Tumour size on day 0}} \times 100
\]

Statistics

All values were expressed as Mean±SEM. The statistical significance of difference between the control and the respective experimental groups was evaluated by Student’s t-test.

Results

1. Recurrent period (Table I)

The recurrence-free survival was extended only in the Bitter Melon group compared to the control. Little difference was observed between the control and the other 3 experimental groups in the length of the recurrent period.

<table>
<thead>
<tr>
<th>Group</th>
<th>Recurrent period(^a) (days)</th>
<th>Mean ± SEM</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (15)(^b)</td>
<td>30.2±7.1</td>
<td></td>
<td>14.9—45.5</td>
</tr>
<tr>
<td>Lily (7)</td>
<td>49.0±17.0</td>
<td></td>
<td>7.3—90.7</td>
</tr>
<tr>
<td>Milk Vetch (2)</td>
<td>42.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bitter Melon (9)</td>
<td>70.2±28.0*</td>
<td></td>
<td>5.5—134.9</td>
</tr>
<tr>
<td>Ginger (9)</td>
<td>38.4±7.8</td>
<td></td>
<td>20.0—56.4</td>
</tr>
</tbody>
</table>

\(^a\)Period in days between the surgical removal of the first tumours and the appearance of secondary tumours. \(^b\)Number of estimates. \(^*\)Significantly different from the control at p<0.05.
2. The growth rate of secondary mammary tumour (Fig. 1)

Mammary tumour growth rate was significantly inhibited in all experimental groups on day 2 after the single trial of GW; however, mammary tumours in groups of Lily + GW, Milk Vetch + GW, and Ginger + GW grew linearly thereafter quite similarly to the control. The tumour growth in the groups of control + GW and Bitter Melon + GW was significantly inhibited during the experiment and the inhibition was somewhat higher in the latter group than the former, while the difference was not statistically significant.

Fig. 1  Growth rate of secondary mammary tumours in each group (Mean). All experimental groups (Lily, Milk Vetch, Bitter Melon and Ginger) and one control received a single trial of whole-body hyperthermia (WBH) with glucose (GW) on day 0. *Mammary tumours which appeared after the surgical removal of the first tumours. *Number of mammary tumours examined. *or** Significantly different from the control at P<0.05 or 0.01. The difference between the groups of Bitter Melon + GW and the control + GW was not statistically significant on any day.
Discussion

The cancer incidence and the life span of both females and males are mostly the lowest and the longest, respectively, in Okinawa, where people consume Bitter Melon more than two times of those in the other prefectures of Japan (Database from Ministry of Health, Labour and Welfare).

The results of the present study revealed that free access to drinking water containing Bitter Melon, which was effective in preventing the development of spontaneous mammary tumors, delayed significantly the appearance of secondary tumors in mice. Furthermore, it did not disturb and somewhat enhanced the inhibition by WBH plus glucose of the growth of secondary tumors. This plant contains much L-ascorbic acid. Ascorbic acid treatment combined with WBH was reported to damage tumor cells, both in vitro and in vivo. This would be at least partly the cause of the influence of Bitter Melon on WBH.

In contrast, the other 3 agents, Gold Banded Lily, Chinese Milk Vetch and Ginger had little effect on the recurrent period. It is generally accepted that tumors which appear after endocrine therapy or chemotherapy are resistant to the therapeutic agent applied previously owing to the selection of tumor cells. This would be the case for these 3 agents, whereas Bitter Melon may be an exception.

No components common to these 3 agents have been specified at present and the mechanism of their disturbance of the inhibitory role of WBH in the formation of secondary mammary tumors remains to be clarified; however, the results strongly suggest that these agents have antagonistic effects to WBH, which are sometimes observed in combination of Chinese medicines.

Incidentally, the combined treatment with WBH and coffee cherry (CC), the residue left after removal of coffee beans from the fruit, each having anti-tumor property, showed no synergistic effects on the inhibition of mammary tumorigenesis, while the body weight loss during WBH was significantly ameliorated by CC. These confirmed the 'normalization effects' of CC usually obtained with Chinese medicine.

Anyway, all findings stress the importance of the therapeutic history of the patient in the efficient application of WBH.

References

マウスの再発乳癌に対する生薬と全身温熱療法の影響

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要　旨：我々は先の実験でヤマユリ、レンゲ、ニガウリ、ショウガがマウス乳癌の予防に効果的であることを明らかにした。本研究では外科的に乳癌を摘出したマウスの再発期間に対するこれら生薬類の有効性、および再発癌に対する全身温熱療法 (WBH) とグルコースの併用 (GW) についてのこれらの生薬の関与を検討した。2ヶ月齢からのニガウリ (0.5%) の飲水による摂取は乳癌の再発期間を延長した。また、ニガウリは GW の乳癌増殖抑制効果をある程度促進させた。一方、ヤマユリ、レンゲ、ショウガは乳癌の再発期間に影響しなかったのみならず、GW の効果を無効にした。これらの結果は WBH を効果的に行うためには、患者のそれまでの抗癌療法に関する十分な情報の必要なことを強く示唆するものである。